

## ESTRUS SYNCHRONIZATION SYSTEMS: GnRH

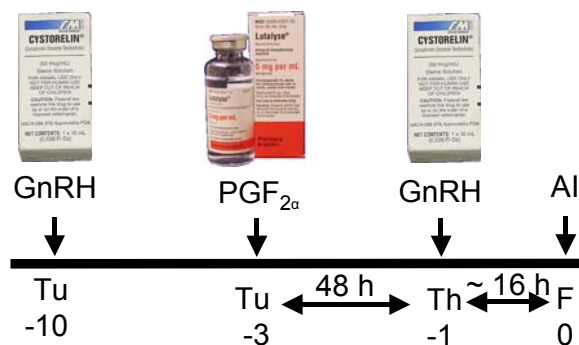
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### Introduction

Development of methods to manipulate the estrous cycle so that all cows are in estrus during a short, predefined period (synchronized estrus) while maintaining normal fertility has been a difficult goal to achieve, but has resulted in the creation of valuable synchronization protocols that are available to producers today. Although implementation of estrus synchronization and AI will improve the profitability of beef operations, no more than 3 to 5% of all beef operations in the U.S. utilize the technology (Patterson et. al., 2001). The major barriers to utilization of estrus synchronization and AI are time and labor (Kesler, 2003).

During the past 25 years, protocols have been developed that minimize time and labor, and yield excellent pregnancy rates. One of the most important steps to creating the wide variety of effective protocols that are available today began with the understanding of follicular waves and the development of the Ovsynch protocol (illustrated in Figure 1). Ovsynch was originally created for use in dairy cattle, however the basic elements (GnRH followed by  $\text{PGF}_{2\alpha}$  seven days later) have as much value in beef cattle. Three protocols (Select Synch, CO-Synch, and Hybrid Synch) have emerged for use in beef cattle and will be discussed within this manuscript.

Figure 1. Ovsynch protocol



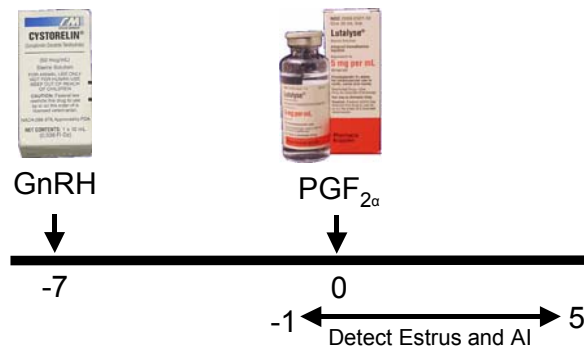
### Select Synch

Select Synch, as well as all of the protocols discussed in this review, includes an injection of GnRH followed by  $\text{PGF}_{2\alpha}$  seven days later. The initial injection of GnRH provokes a preovulatory-like LH surge (Pursley et al., 1995). Studies have demonstrated that this single injection of GnRH induces ovulation in most cows, including >80% of late-calving anestrus

cows suckling calves (Thompson et al., 1999). A new follicular wave is then initiated about two days after the GnRH-induced ovulation (Kojima and Patterson, 2003). There are a number of GnRH products available and all seem to have similar efficacy, assuming a full 100 mcg dose is administered. More variable responses, including decreased efficacy, have been reported when cows are administered a half dose of GnRH (John B. Hall, personal communications). Furthermore, 18 g needles that are 1.5 inches long are recommended and GnRH and PGF<sub>2</sub>α should be injected intramuscularly in the neck.

Seven days after the injection of GnRH cows are administered an injection of PGF<sub>2</sub>α to induce regression of corpora lutea, if present. Although 25-33% of the estrus-cycling cows will not have corpora lutea and do not need the PGF<sub>2</sub>α, it is not efficient to attempt to differentiate cows with corpora lutea from those without. Therefore, all cows should receive an injection of PGF<sub>2</sub>α seven days after the GnRH injection. The protocol is illustrated in Figure 2.

**Figure 2. SelectSynch protocol**



Cows synchronized with the Select Synch protocol are bred based upon the detection of estrus. The majority of cows will exhibit estrus 36 to 72 hours after PGF<sub>2</sub>α (Stevenson et al., 2000). However, a small percentage will exhibit estrus outside this peak period (see Figure 3), including 8 to 10% that show estrus prior to the injection of PGF<sub>2</sub>α (Geary et al., 2000). Furthermore, not all cows are detected in estrus—ranging from 7 to 61% in published data. We recommend that estrus detection begin the day before injecting PGF<sub>2</sub>α followed by 4 to 7 days of estrus detection—including the day PGF<sub>2</sub>α is administered. Although the injection of GnRH may induce the first postpartum ovulation and hasten conception, fertility in cows in poor body condition will still be low (Stevenson et al., 2000; see Table 1).

**Figure 3. Distribution of estrus after SelectSynch**

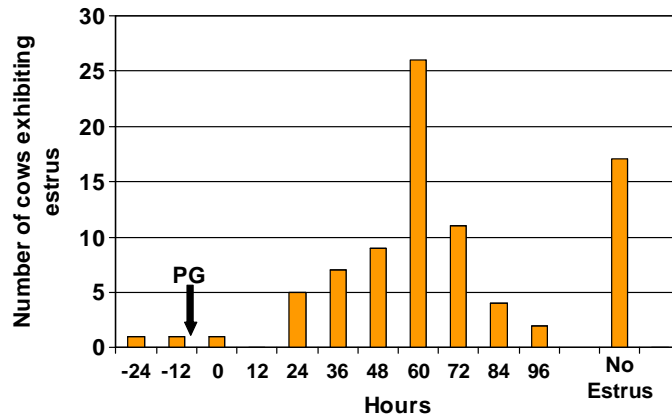


Table 1. Pregnancy rates in suckled beef cows after treatment with Select Synch

Body Condition	Select Synch
4.0 or less	28%
4.5	39%
5.0 or greater	50%

The Select Synch procedure was developed for operators who do not object to, or feel more comfortable with, breeding upon the detection of estrus. The Select Synch protocol has been effectively utilized with very encouraging results as reported in Table 2. As shown in Table 2, estrus detection rates and pregnancy rates are highly correlated ( $r = .96$ ;  $P < .01$ ). Low responses may be due to compromised estrus detection efficiency, postpartum anestrus, or a combination of both. However, it does illustrate the importance of estrus detection and of using this protocol only when one is fully committed to thorough monitoring of estrus.

Table 2. Estrus response rates and pregnancy rates in cows administered the Select Synch protocol

Study	Estrus Response	Pregnancy Rate
Kojima et al., 2000	69%	47%
DeJarnette et al., 2001a:		
experiment 1	93%	70%
experiment 2	78%	52%
Stevenson et al., 2000:		
experiment 1	59%	38%
experiment 3	63%	44%
Patterson et al., 2001	67%	45%
Constantaras et al., 2004	80%	65%

## CO-Synch

The CO-Synch protocol utilizes the same strategy as Select Synch; however, it uses a single fixed time AI. The protocol is illustrated in Figure 4. No estrus detection is required with CO-Synch—a major attribute of this protocol. Like Select Synch, cows must be in good body condition as results are compromised in cows in poorer body condition, as illustrated in Table 3 (Lamb et al., 2001).

**Figure 4. CO-Synch protocol**

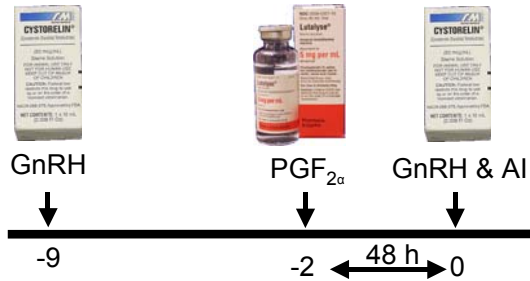


Table 3. Pregnancy rates in suckled beef cows after treatment with CO-Synch

Body Condition	Select Synch
4.5 or less	30%
4.5 to 5.0	41%
5.5 or greater	59%

The CO-Synch protocol has been used in a large number of diverse situations quite successfully. Table 4 is a summary of the available published data where CO-Synch was used. Overall, pregnancy rates have average 48%. The protocol is quite simple to employ as all injections and timed AI can be done at the same time of the day. However, details must be followed closely. In the study by Larson et al. (2004) cows were bred at 54 hours after the injection of PGF<sub>2α</sub>, by design in this case, and pregnancy rates were compromised.

Table 4. Pregnancy rates in cows administered the CO-Synch protocol

Study	Pregnancy Rates
Geary et al., 1998:	
cyclic cows	59%
anestrus cows	49%
Geary et al., 1998:	
location 1	49%
location 2	52%
location 3	46%
Stevenson et al., 2000	33%
Geary et al., 2001	49%
Geary et al., 2001	54%
Stevenson et al., 2003:	
experiment 1	61%
experiment 2	31%
Lamb et al., 2001:	
location 1	52%
location 2	54%
location 3	38%
location 4	53%
Perry et al., 2001	47%
Larson et al., 2004	43%
Constantaras et al., 2004	48%

Some have speculated that short-term calf removal, from the time of PGF<sub>2</sub> $\alpha$  until AI is completed, may improve pregnancy rates. Geary and co-workers (2001) examined this concept and demonstrated an improvement in one experiment, but not another as illustrated in Table 5. Similar results were observed when short-term calf removal was used with Syncro-Mate B. It is important to note that in order to utilize short-term calf removal one must have excellent holding facilities.

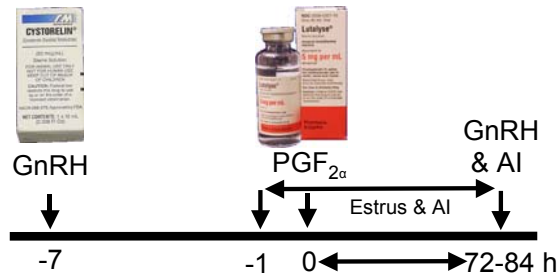
Table 5. Effect of short-term calf removal on pregnancy rates of cows synchronized with CO-Synch

Study	Pregnancy Rates
Geary et al., 2001:	
with calves	54%
calf removal	63%
Geary et al., 2001:	
with calves	49%
calf removal	46%

## Hybrid Synch

Hybrid Synch, as the name implies, is a blend between Select Synch and CO-Synch. This procedure was created to optimize pregnancy rates in cows administered GnRH-PGF<sub>2α</sub> protocol. Because the interval from PGF<sub>2α</sub> to estrus is variable, as illustrated in Figure 3, it is impossible to select a single time that all cows have an excellent opportunity to conceive. Therefore, the insemination time for CO-Synch is the single time expected to achieve the highest pregnancy rate—not the optimum time when each individual has the best opportunity to conceive. In order for more cows to have an opportunity to conceive one may breed upon the detection of estrus for a period of time followed by a clean up timed AI—the Hybrid Synch protocol (illustrated in Figure 5). Upon examination of Figure 3, one will note that the highest percentage of cows in this study were in estrus at 60 hours after the PGF<sub>2α</sub> injection.

**Figure 5. HybridSynch protocol**



Therefore, the ideal time for clean up timed AI for the majority of the cows is 72 hours. In the Hybrid Synch protocol it is recommended that the clean up timed AI be done at 72 to 84 hours after PGF<sub>2α</sub>. This clean up timed AI is only for cows not previously detected in estrus. Furthermore, cow detected in estrus do not need an injection of GnRH at insemination. However, cows at the clean up timed AI should be concurrently administered an injection of GnRH. This will improve the likelihood that ovulation will be synchronized with the insemination. Results from published data are summarized in Table 6.

Table 6. Pregnancy rates in cows administered the Hybrid Synch protocol

Study	Estrus Response	Pregnancy Rates
Stevenson et al., 2000	19%	34%
DeJarnette et al., 2001b:		
experiment 1	44%	44%
experiment 2	74%	47%
Larson et al., 2004		53%
DeJarnette et al., 2004:		
herd A-01	75%	51%
herd A-02	60%	44%
herd B-F-01	100%	71%
herd C-00	75%	67%
herd C-01	23%	23%

The results are variable (overall average of 48% [data in Table 6]) and don't appear considerably higher than for Select Synch (overall average of 52% [data in Table 2]) and CO-Synch (overall average of 48% [data in Table 4]); however, it will allow one to maximize the opportunity for obtaining the greatest overall pregnancy rates. Similar to results in Table 2 for Select Synch, the estrus response was correlated ( $r = .90$ ;  $P < .01$ ) to pregnancy rates. Again this suggests that poor estrus detection and/or postpartum anestrus compromised efficacy. Some have even suggested that if the estrus response before the timed AI is poor, following up with the timed AI should be reconsidered.

### **Select Synch + ReCycleSynch**

Because not all cows are inseminated in the Select Synch protocol, cows not detected in estrus and inseminated may be resynchronized for a second breeding. This potentially reduces the time to conception and allows for utilization of AI. This procedure was used on a group of cows by administering CO-Synch beginning six days after the original injection of PGF<sub>2</sub> $\alpha$  to cows that were not observed in estrus and inseminated. Because we started breeding the day before PGF<sub>2</sub> $\alpha$  we had a 16-day breeding period. Pregnancy rate at the end of the Select Synch protocol was 65% (Constantaras et al., 2004). With the additional cows conceiving to the CO-Synch protocol, the 16 day AI breeding pregnancy rate was 78%. This is only a slight increase in drug cost as only the cows that were not inseminated after Select Synch were administered CO-Synch; however, there is a significant increase in time and labor.

### **Heifers**

Early studies concluded that GnRH-based protocols with timed AI (Ovsynch and CO-Synch) should not be used in heifers. For example, Martinez et al. (2002) reported pregnancy rates of 39% in heifers synchronized with CO-Synch. This compares to a 68% pregnancy rate in heifers synchronized with a CIDR-based system in the same study (Martinez et al., 2002) and an average 56% pregnancy rate for heifers synchronized with an MGA-based system (14 days of MGA followed by PGF<sub>2</sub> $\alpha$  19 days after the last day of MGA feeding; Kesler, 2003) in other studies. More recently, Select Synch has been successfully used in heifers with very good fertility. Lamb et al. (2004) conducted a multi-herd study: heifers were administered Select Synch, two injections of PGF<sub>2</sub> $\alpha$ , or the MGA-based system. A greater percentage of MGA treated heifers (83%) were detected in estrus during the target-breeding week than for Select Synch and PGF<sub>2</sub> $\alpha$  treated heifers (74% and 75% respectively). Most of the heifers displayed estrus between 24 and 72 hours. The peak period for Select Synch treated heifers was between 24 and 48 hours after PGF<sub>2</sub> $\alpha$ , whereas the peak period for the MGA treated heifers was between 48 and 72 hours. Conception rates ranged from 63 to 68% and pregnancy rates ranged from 47% to 56% and were not different. Funston et al. (2004) also conducted a multi-herd study. They similarly demonstrated that the MGA-based protocol was more effective in synchronizing estrus; however, conception rates and overall AI pregnancy rates for the MGA-based protocol and Select Synch were similar. Combined, these data suggest that Select Synch will effectively synchronize estrus in heifers; however, attempting to time AI is not recommended at this time.

## Follicular Dynamics

Research to further understand and/or improve the efficacy of these protocols continues. Follicular dynamics are of particular interest. The use of GnRH at the time of insemination results in a wide range of follicle sizes being ovulated (Perry et al., 2003). Lamb et al. (2001) demonstrated that pregnancy rates increased as follicular size at the time of second GnRH injection (for the CO-Synch protocol) increased to 16.0 to 17.9 mm and then dropped. Furthermore, Mussard et al. (2003) demonstrated that when embryos of similar quality were transferred into cows induced to ovulate small (< 12 mm) or large (> 12 mm) follicles, pregnancy rates were significantly higher in cows that ovulated with large follicles. Therefore, the goal in a timed AI protocol is to administer the second GnRH injection at a time when cows have large follicles, yet before spontaneous ovulation—a difficult goal to achieve.

## Estrogens

It is important to point out that some scientists have reported that the use of estrogen—estradiol and estradiol benzoate—may improve synchronization efficacy; however, extensive multi-location studies do not exist. The consensus of many, including most of the scientists with reports at this workshop, agree that estradiol use should be suspended at this time. This recommendation is based upon a study that reported a higher incidence of invasive breast cancers in women administered a postmenopausal estrogen/progestin product (Women's Health Initiative, 2002). Estrogens will certainly cause breast cancers to proliferate; however, is it a cause of breast cancer? The Women's Health Initiative study convinced the public, including a high percentage of physicians, that estrogens cause breast cancer. A smaller arm of the Women's Health Initiative (2002)—that did not receive significant publicity—was the study where estrogen alone was used in women with hysterectomies. In this study, there was no evidence that estrogen caused cancer (Nelson et al., 2002). However, there is considerable public concern and we do not need to further concern the public with the safety of the product beef producers provide. Besides, estradiol and estradiol benzoate are not approved by FDA for this use. Hence, it is not an extra-label use—it is illegal to use estradiol or estradiol benzoate to synchronize estrus and ovulation. The only estrogen approved for use in cattle is estradiol cypionate (ECP<sup>®</sup>). Studies using ECP<sup>®</sup> in beef cattle have been conducted and a protocol (Heat Synch) has been used in dairy cows. However, because of the public concern with estrogens availability of ECP<sup>®</sup> in the future is questionable. Protocols discussed in this review are therefore recommended unless a protocol utilizing ECP<sup>®</sup> is in place, but be prepared to shift to another protocol.

## Efficacy of Different GnRH Products

The efficacy of the specific GnRH product used with the Select Synch, CO-Synch, and Hybrid Synch protocols has been discussed. Much of the discussion was caused by a study published by Martinez et al., (2003). Martinez et al. (2003) reported that Cystorelin<sup>®</sup> provoked a greater LH surge than Fertagyl<sup>®</sup> and Factrel<sup>®</sup>. Similarly, Cystorelin<sup>®</sup> induced a higher ovulation rate; however, all products synchronized follicular wave emergence. GnRH is a decapeptide—a linear chain of ten amino acids. The base for Cystorelin<sup>®</sup>—and Fertagyl<sup>®</sup> (and Ovacyst<sup>™</sup> another GnRH product not included in the Martinez study)—is diacetate, tetrahydrate. Therefore, Cystorelin<sup>®</sup>, Fertagyl<sup>®</sup>, and Ovacyst<sup>™</sup> are chemically identical. Factrel<sup>®</sup> has a HCl base which



should not alter bioactivity. If the GnRH products are chemically identical, then why did Martinez et al. (2003) observe differences? Being quite familiar with pharmaceutical manufacturing we realize that companies are permitted to include a wide range of active compound in the product. It is unknown if the company manufactures at the low or high end of this range. Hence, the results of Martinez et al. (2003) may only be a difference in active GnRH within the product. One must remember, the dose was selected based on the treatment of cystic ovarian disease—the clinical claim for GnRH products. This raises a previously mentioned point. One should use a full dose of GnRH as more variable responses, including decreased efficacy, has been reported when cows are administered a half dose of GnRH (John B. Hall, personal communications). Although all dominant follicles ( $\geq 10$  mm) have the ability to ovulate in response to a GnRH-induced LH surge, Sartori et al. (2001) demonstrated that a larger dose of LH was required to induce ovulation of a 10 mm follicle compared to larger follicles. Certainly, this subject needs further study.

### Implications

The purpose of this article is to review the GnRH-based estrus synchronization protocols. A succinct summary is provided in the following table (Table 7).

Table 7. GnRH/PGF<sub>2</sub> $\alpha$ -based estrus synchronization protocols used in beef cows

Protocol	Description
Select Synch	<ul style="list-style-type: none"> <li>• The duration of the protocol is only one week; however, breeding should begin six days after initiating the protocol because a percentage of cows exhibit estrus before the injection of PGF<sub>2</sub><math>\alpha</math>.</li> <li>• This protocol requires minimal drug cost; however, considerable time is required for detection of estrus.</li> <li>• In order for this protocol to be successful, estrus detection must be emphasized. With emphasis on estrus detection, one can obtain excellent pregnancy rates if cows are in good body condition.</li> <li>• AI pregnancy rates may be improved if cows not detected in estrus are subsequently administered CO-Synch.</li> </ul>
CO-Synch	<ul style="list-style-type: none"> <li>• The duration of this system is nine days.</li> <li>• Because this is a timed AI protocol and all cows are inseminated 48 hours after the injection of PGF<sub>2</sub><math>\alpha</math>, it does not require the time and labor associated with detecting estrus.</li> <li>• At the time of AI, cows are also administered an injection of GnRH which increases the drug cost as compared to Select Synch; however, time and labor are minimized.</li> </ul>
Hybrid Synch	<ul style="list-style-type: none"> <li>• This is a blend of Select Synch and CO-Synch protocols and maximizes the opportunity for obtaining the greatest overall pregnancy rates.</li> <li>• Cows are bred upon the detection of estrus for the first 72-84 hours. Then any cow not detected in estrus is administered GnRH and inseminated. Drug costs are reduced as compared to CO-Synch as cows detected in estrus are not administered GnRH at AI. However, labor costs are increased as compared to CO-Synch.</li> </ul>

Other scientists are summarizing results utilizing progestins (MGA- and CIDR-based systems) and can be found elsewhere in these proceedings. Although the progestin-based systems may have higher pregnancy rates in some situations, the GnRH-based systems without progestins have value. In fact, a supermarket of estrus synchronization protocols for producers with different needs exists today. Three of the protocols within this estrus synchronization supermarket are Select Synch, CO-Synch, and Hybrid Synch. These are systems minimizing drug costs compared to some others; however, cows must be in good body condition and postpartum anestrus may compromise efficacy.

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